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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Richard A. Goldsby et al.	Art Unit:	1632
Serial No.:	09/714,185	Examiner:	Not Yet Assigned
Filed:	November 17, 2000	Customer No.:	21559
Title:	PRODUCTION OF UNGULATES, PREFERABLY BOVINES THAT PRODUCE HUMAN IMMUNOGLOBULINS		

Assistant Commissioner For Patents
Washington, DC 20231

Version with Markings to Show Changes Made

Marked-up versions of claims 1, 2, 6, 7, and 15 are presented below.

1. (Amended) A method for producing a cloned ungulate wherein the expression of both copies of a gene essential for B cell production has been knocked out, wherein said gene is [selected from the group consisting of Igα,] IgM, [E2A, EBF, BSAP, rag-1 and rag-2], which comprises the following steps:

(i) producing a male and/or female ungulate cell wherein the expression of one or both copies of the [Igα, E2A, EBF, BSAP,] IgM heavy chain[, rag-1 and/or rag-2 gene] has been eliminated by targeted disruption;

(ii) using said cell or DNA therefrom as a donor for nuclear transfer by fusing or inserting said donor cell or nucleus into an oocyte or

blastomere, which is enucleated before or after transfer, activating the resulting nuclear transfer unit and/or the oocyte prior or simultaneous to nuclear transfer and culturing in a suitable medium to produce a nuclear transfer embryo;

(iii) introducing said nuclear transfer embryo into a female ungulate; and

(iv) obtaining a cloned fetus or animal ungulate that expresses the genotype of the donor differentiated cell, in which one or both copies of the IgM (mu) chain[, Igα, E2A, EBF, BSAP, rag-1 and/or rag-2] gene have been eliminated: and

(v) optionally, mating said cloned male or female ungulate with another cloned female ungulate wherein one copy of the IgM[, rag-1 or rag-2] gene has been knocked out and selecting progeny wherein both copies of the [Igα, E2A, EBF, BSAP,] IgM[, rag-1 or rag-2] genes have been knocked out.

2. (Amended) The method of Claim 1, wherein the expression of both copies of the [E2A, Igα, EBR, BSAP,] IgM heavy chain (mu)[, rag-1 and/or rag-2] gene is eliminated, by a three-step process comprising the following steps:

(i) a desired ungulate cell is contacted with a DNA construct that provides for targeted deletion or inactivation of said [Igα,] IgM (mu)[, E2A, EBF, BSAP, rag-1 or rag-2] gene by homologous recombination;

(ii) the resulting differentiated cell or DNA therefrom, wherein the expression of one copy of the [Igα,] IgM[, EBF, E2R, BSAP, rag-1 and/or rag-2] gene has been knocked out, is used as a nuclear transfer donor and is fused or inserted into an enucleated oocyte;

(iii) the resulting nuclear transfer unit is allowed to develop into an embryo, and a cell is obtained from this embryo and is contacted with a second DNA construct under conditions that results in the elimination of the expression

of the other (second) copy of the IgM[, Igα, E2A, EBF, BSAP, rag-1 and/or rag-2] gene; by homologous recombination; and

(iv) the resulting cell, in which both copies of the [Igα,] IgM (mu)[, E2A, EBF, BSAP rag-1 and/or rag-2] gene have been knocked out, is used as a nuclear donor for nuclear transfer by fusing or inserting said donor cell or DNA therefrom into an enucleated oocyte or blastomere, activating the resultant nuclear transfer unit after oocyte prior to nuclear transfer, and culturing in a suitable medium to produce a nuclear transfer embryo which does not express [E2A, EBF, BSAP, Igα,] IgM heavy chain[, rag-1 or rag-2].

6. (Amended) The method of Claim 1, wherein the differentiated cell of (i) is produced by sequentially contacting said cell with two knockout constructs which in combination provide for knockout of both copies of the [Igα, E2A, EBF, BSAP,] IgM gene [, rag-1 and/or rag-2 genes].

7. (Amended) The method of Claim 6, wherein the said two knockout constructs comprise different selectable markers thereby providing for the selection of cells wherein both copies of the IgM heavy chain[, rag-1 and/or rag-2] are eliminated.

15. (Amended) A transgenic ungulate wherein both copies of the IgM heavy chain (mu)[, rag-1 and/or rag-2 gene] have been knocked out.

REMARKS

Claims 1, 2, 6, 7, and 15 have been amended to remove references to Igα, E2A, EBF, BSAP, rag-1, and rag-2. Applicants intend to pursue the cancelled subject matter in a related divisional application.

The specification was also amended to indicate that this application claims priority to U.S.S.N. 60/166,410, filed November 19, 1999. Applicants note that the Combined Declaration and Power of Attorney filed with the application listed U.S.S.N. 60/166,410 as a priority application.

No new matter is introduced by these amendments.

CONCLUSION

Applicants submit that this application is now in condition for allowance, and such action is respectfully requested. A marked-up version indicating the amendments made to the claims, as required by 37 C.F.R. § 1.121(c)(1)(ii), is enclosed.

If there are any charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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